TRANSCRIPTIONAL REPRESSOR PEPTIDES AND GENES FOR THE SAME

TECHNICAL FIELD

[0001] The present invention relates to (i) a peptide which is capable of repressing transcription, (ii) a gene encoding the peptide, (iii) a chimeric protein which is capable of repressing transcription, the chimeric protein including the peptide and a transcription factor or its DNA-binding domain, the peptide binding to the transcription factor or its DNA-binding domain, (iv) a chimeric gene encoding the chimeric protein, (v) a recombinant vector including the chimeric gene, and (vi) a transformant including recombinant the vector.

BACKGROUND ART

[0002] The antisense method and the ribozyme method are conventionally known as means of repressing expression of organisms' genes. These techniques have been studied so as to be applied to, e.g., repression of expression of disease-causing genes (such as oncogenes) or improvement of plants. The antisense method employs antisense DNA or RNA that is complementary to a specific site of a target gene, transcription of which is to be repressed, or mRNA to which the target gene has been transcribed. The prepared antisense DNA or RNA, however, cannot be used to repress expression of genes other than the target gene. Thus, for other genes, it is necessary to all the way prepare antisense DNA or RNA in accordance with sequences of the respective genes. In the case of the ribozyme method, in order to cleave target DNA or mRNA with a ribozyme, the ribozyme must be designed (i) to have a sequence complementary to the target DNA or mRNA so as to bind thereto and (ii) to cleave the target DNA or mRNA at a predetermined position. Even if the ribozyme is designed to cleave the target gene, the following problem may occur: For example, in a case where such the ribozyme is linked to a promoter (e.g., the cauliflower mosaic virus 35S promoter) and a transcription terminator sequence in order to construct a vector for introduction and the resulting vector is actually introduced to a plant cell, an excessive sequence is added to a transcribed ribozyme, which may result in loss of ribozyme activity. In addition, in these conventional techniques, identification of the target gene and determination of its nucleotide sequence are always indispensable. This has been a big problem, particularly when these techniques are used for improvement in plant traits. The reason for this is that most studies on plants have been conducted with use of model plants, and there is little finding on gene sequences of practical plants which are used as food, fuels, building materials, etc., and therefore it is very difficult to design appropriate antisense DNA or RNA or an appropriate ribozyme. Moreover, it is well known that the practical plants have big differences in their gene sequences between the types, or even between the individuals. In view of this, it is almost impossible to design appropriate antisense DNA or an appropriate ribozyme for each type of the practical plants. In addition to the above-described methods, as a technique for repressing expression of a target gene, there is a method for disrupting a target gene itself by a gene knock-out method, which disrupts an endogenous gene by a chemical treatment, radiation, or introduction of a foreign gene. However, this method is difficult to apply to, e.g., amphidiploid plants, which inherently have a large number of gene sets, since it is difficult to disrupt all of the genes of the amphidiploid plants by this method. Examples of polyploid plants encompass soybean and wheat, each of which is an important crop as food and animal feeding stuff. In plants, genes often redundantly exist for an important function. Thus, also in cases of general diploid plants, it is difficult to disrupt all genes by the gene knock-out method.

[0003] In order to address these problems, the present inventors have developed CRES-T (chimeric repressor silencing technology), which is a completely different approach from the above conventional techniques (see Patent Literatures 1 through 8 and Non-Patent Literatures 1 through 3). The CRES-T is a technique that uses a transcriptional repression domain (dominant repressor) isolated from a plant. Namely, according to the CRES-T, such the transcriptional repression domain is bound to the carboxyl terminus of a transcriptional activator so as to impart strong transcriptional repression activity to the transcriptional activator, and then a chimeric gene including nucleic acid molecules encoding the transcriptional repression domain and the transcriptional activator is expressed in a plant, so that transcription of a target gene is strongly repressed. Further, such the chimeric gene, to which the transcriptional repression domain has fused, represses not only the transcriptional activator but also functions of all other transcriptional activators that function redundantly with respect to the same gene. Therefore, plants produced by the CRES-T exhibit traits resulting from complete repression of expression of the target gene. Thus, the CRES-T is very useful not only for transformation of the practical plants but also for unravelment of basic functions of genes. Furthermore, the CRES-T can repress functions of a related gene whose sequence and functions are analogous to those of the target gene. Therefore, unlike the conventional antisense method and ribozyme method, the CRES-T does not need to design DNA or RNA according to a nucleotide sequence of each target gene. Thus, the CRES-T can be carried out in a simple manner, and is widely applicable.

[0004] The transcriptional repression domain consists of a motif (L/F)DLN(L/F)(X) P, where X represents any amino acid residue. At first, the transcriptional repression domain was isolated from *Arabidopsis thaliana*, and therefore the studies were conducted mainly on *Arabidopsis thaliana*. Afterward, the same motif as above was identified in transcriptional repressors of a wide variety of plants, examples of which include *Nicotiana tabacum* and monocotyledons such as *Oryza sativa*. So far, it has been demonstrated that, by converting a transcription factor functioning in the secondary metabolism biosynthesis system into a chimeric transcriptional repressor, it is possible to actively regulate the secondary metabolism biosynthesis. Further, the following experimental result has been obtained: By converting a transcription factor that regulates formation of floral organs into a chimeric transcriptional repressor and causing the chimeric transcriptional repressor to express in plants, male sterility and complete sterility were successfully induced with a high probability not only in *Arabidopsis thaliana* but also in *Oryza sativa*. These results show that the CRES-T is also applicable to *Oryza sativa*, which is a monocotyledon. Thus, the CRES-T attracts attention as a revolutionary technology that can be applied to a wide variety of general plants.

[0005] However, not only for plants but also for overall living organisms, the motif (L/F)DLN(L/F)(X) P is the only conserved motif which has been found so far to be the transcriptional repression domain. After the finding of the motif (L/F)DLN(L/F)(X) P, the present inventors found that the motif "DLELRL" is included in publicly-known SUP gene as